Novel Multi-Herbal Formulation (SS-IM-21) and its Sanative Effect in CCl_4-Induced Experimental Animals

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ABSTRACT

The nutritional status can be determined by serum proteins. Normal protein levels being off-balance signals possible life-threatening organ malfunction. The study's primary goal was to ascertain the serum and tissue protein levels in response to CCl_4 intoxication and their reduction by a newly created innovative herbal medicine (SS-IM-21). Swiss albino adult healthy mice were divided into four groups of six each based on weight. Group I served as the control, group II was given multi-herbal formulation (SS-IM-21) (400 mg/kg/day), group III was given carbon tetrachloride (CCl_4) (1 ml/kg-bw), and group IV was given CCl_4 combined with SS-IM-21 (400 mg/kg). In contrast to untreated animals, therapy with Multi herbal formulation (SS-IM-21) normalized body weight, food intake, and water intake in mice after administration of carbon tetrachloride (CCl_4). When compared to the control, group III receiving CCl_4 treatment saw lower serum levels of total protein, albumin, and globulin. In comparison to CCl_4-impaired animals, pre-treatment with SS-IM-21 significantly ($P < 0.001$) raised the levels of total protein, albumin, and globulin in serum, liver, and kidney. However, a greater albumin/globulin ratio makes it evident that the liver and kidney may be negatively impacted by CCl_4 medication. By preserving the albumin/globulin ratio, the multi-herbal formulation (SS-IM-21) safeguards the kidney and liver. In conclusion, it is possible to predict that antioxidant-enhanced new formulations with phytochemicals preserve normal protein patterns and shield the organism from a variety of dysfunctions.

Key Words: *Swiss albino mice, Multi-herbal formulation, Serum protein, Albumin, Globulin*

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INTRODUCTION

Protein is necessary for the body to survive and operate. A change in serum total protein causes several issues and can occasionally harm important organs [1]. Albumin and globulin, which make up the majority of the serum protein, represent the body's nutritional condition and keep the blood's colloidal osmotic pressure in check [2-4]. Additionally, these two proteins support the body's immune system and guard against infection [5]. Albumin-to-globulin ratio (AGR) has been shown in scientific literature to be a predictive factor for some diseases and medical issues [6, 7]. Clinical research suggests that declining albumin levels indicate inadequate dietary status, which could occasionally be lethal to survival [8, 9].

A significant industrial contaminant, carbon tetrachloride (CCl_4) is linked to the formation of free radicals, which can lead to liver and kidney damage, among other organ dysfunctions [10]. It is known that cytochrome P450 metabolic activation of CCl_4 produces trichloromethyl radicals (CCl_3) and peroxy trichloromethyl radicals ($OOCCl_3$) that cause membrane rupture and cause harm to the liver and kidneys [11]. Long-term CCl_4 exposure alters the body's natural protein levels, leading to a variety of organ dysfunctions [12]. According to an animal investigation, the injection of CCl_4 causes nutrition deficiency syndrome by reducing normal food and water intake [13].

One of the key approaches of an alternative system of medicine that uses herbal medicines is long-term safe and symptomatic medication without side effects [14]. The

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plant-based formulation is enhanced with a wide range of crucial phytochemicals and significant antioxidants, serrates, and other disease-prevention agents [15, 16]. We created a brand-new herbal product that is inexpensive and made of healing herbs and spices. Our earlier research on animals demonstrated that this formulation is safe for therapeutic medicine and has no adverse effects [17, 18]. Here, we attempt to employ this conventional therapy to preserve the critical protein levels brought on by CCl_4.

MATERIALS AND METHODS

Reagents and chemicals

TRIS buffer and carbon tetrachloride (CCl_4) were purchased from Merck in India. We purchased PBS pH 7.4 from Sigma-Aldrich. Total protein, albumin, and globulin biochemical measurement kits were purchased from Thermo Scientific in the United States. Laboratory-grade materials were employed for all other reagents in this study.

Extraction of plant material

All of the medicinal plant and spice components were procured from licensed local herbal vendors and verified by pharmacognosists. The portions of the plants were cleaned and dried at room temperature. According to a tried-and-true methodology, dried plant components were employed to create a multi-herbal mixture [19]. **Table 1** provides a list of the plants and plant components employed in the extraction process.

Table 1. Details of ingredient(s) present in the newly developed multi-herbal formulation (SS-IM-21), 5 ml extract contains the following ingredients

Sl. No.	Scientific Name	Common Name	Family	Quantity
1.	*Andrographis paniculata*	Kalmegh	Acanthaceae	50 mg
2.	*Withania somnifera*	Ashwagandha	Solanaceae	25 mg
3.	*Ocimum sanctum*	Tulsi	Lamiaceae	50 mg

Animals

For the investigation, 24 young, healthy, Swiss albino mice weighing 25 ± 5 g were randomly selected. The animals have been kept in a safe environment with normal feeding, drinking, and medical care following CPCSEA recommendations. This includes 12 hours of light and dark cycles, at 25 °C and 50–60% humidity. Mice were maintained under close supervision for a week before the experiment's start to acclimatize and rule out any undetected infections. The Institutional Animal Ethics Committee (IAEC) gave its approval to the experimental methods (Approval No. 261/Dey's/IAEC/Pharma/2018).

Experimental technique

According to their body weights, the mice were randomly divided into four large groups of six mice each, ensuring that each group contained animals with similar body weights. These are the groups: group-I is used as the control group, group-II is given Multi herbal formulation (SS-IM-21) at a dose of 400 mg/kg per day, group-III is given carbon tetrachloride (CCl_4) at a dose of 1 ml/kg-bw, and group-IV is given CCl_4 combined with SS-IM-21 (400 mg/kg).

Body mass, calorie intake, and water intake

From the first day of the experiment until the last day, body weights were recorded weekly to determine body weight change. Measuring feed residue weekly since the start of the experiment allowed for the determination of feed consumption. Total feed consumption was divided by body weight growth to calculate feed conversion. The amount of water consumed was calculated by deducting the amount of water still in the drinking bottle from the amount of water that was first given to the animals.

Blood collection and production of serum

Blood was drawn from each mouse by a retroorbital venous puncture after the appropriate fasting period. 200 µL of blood was drawn and placed in 2% EDTA-containing microcentrifuge tubes. Blood samples were collected and left at room temperature for two hours in a slanting position. They were then centrifuged for 10 minutes at 3500 g. The serum of a clear, light yellow tint was extracted and used for more research.

Preparation of tissue homogenate

To obtain 20% homogenate, a tiny amount of the liver and kidney tissues were homogenized in ice-cold 0.9% w/v saline using a homogenizer. Before biochemical analysis, aliquots of the liver homogenate were kept at -4 °C.

Serum, liver, and kidney protein determination

Total protein, albumin, and globulin were measured using tissue homogenate and serum. Using a colorimetric kit purchased from Thermo Scientific, USA, total protein, albumin, and globulin were measured following the standard biochemical methodology with a minor modification.

Statistic evaluation

The data are shown as mean ± SE. Two-way analysis of variance (ANOVA) was used to statistically analyze the

data, and Tukey's test was used for post hoc analysis. A level of 0.05 was considered acceptable for statistical significance.

RESULTS AND DISCUSSION

A multi-herbal supplement (SS-IM-21) impacts on body weight, food intake, and water intake

Table 2 displayed gross body weights, relative changes, food intake, and water intake. When compared to control mice, the administration of carbon tetrachloride (CCl_4) (1 ml/kg-bw) significantly ($P < 0.001$) decreased body weight, food intake, and water intake capacity. In comparison to control animals, treatment with multi-herbal formulation (SS-IM-21) 400 mg/kg/day normalized body weight, daily food intake, and water intake capacity and decreased liver weight. When SS-IM-21 was administered to animals, there were no aberrant differences from control animals.

Table 2. Effect of multi-herbal formulation (SS-IM-21) on body weight, food consumption, and water intake

Parameters	Mice			
	Group-I	Group-II	Group-III	Group-IV
Body weight (g) Initial	25.85 ± 1.96	26.11 ± 2.15	25.71 ± 4.24	26.08 ± 5.2
Body weight (g) Final	38.81 ± 2.07	36.59 ± 1.61	20.71 ± 2.61$^{\#}$	37.92 ± 1.87*
Body weight (g) gain or loss	12.96 ± 0.07 (+)	10.48 ± 0.05 (+)	5.00 ± 0.008 (-)	11.12 ± 0.04 (+)
Food consumption (g)	4.52 ± 0.05	4.37 ± 0.07	2.94 ± 0.06$^{\#}$	5.11 ± 0.04*
Water intake (ml)	4.01 ± 0.04	4.25 ± 0.04	3.01 ± 0.02$^{\#}$	4.31 ± 0.06*

All data were expressed as mean ± SE (n = 6/group). (+) Body weight gain and (-) Body weight loss. Data comparison was performed using two-way ANOVA followed by Tukey's Multiple Comparison Test. $^{\#}$Significantly different from the control group at $P < 0.001$ and *Significantly different from (CCl_4) group values at $P < 0.001$

A multi-herbal supplement (SS-IM-21) effects on serum, liver, and kidney total protein

The mean serum, liver, and kidney total protein (TP) concentrations in the control and experimental groups of mice are shown in **Figure 1**. The results show that the mean blood levels of liver and kidney total protein in CCl_4-intoxicated mice were considerably lower than those in the control group ($P < 0.001$). When compared to mice treated with CCl_4, pre-treatment with the multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day significantly exacerbated the decline in total protein levels. When compared to the control group, 28 days of treatment with the newly created multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day did not demonstrate any appreciable variations in serum, liver, or kidney protein levels.

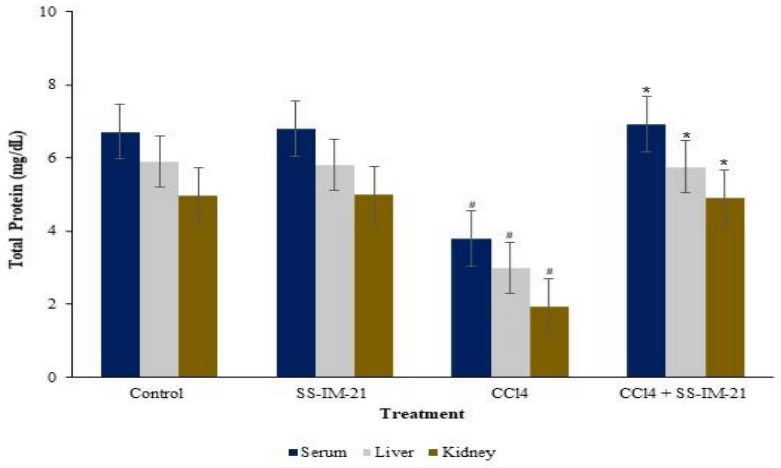

Figure 1. Effect of newly developed multi-herbal formulation (SS-IM-21) on Total protein levels in mice. All data were expressed as means ± SE (n = 6/group). $^{\#}$Significantly different from the control group at $P < 0.001$ and *Significantly different from (CCl_4) group values at $P < 0.001$. Data comparison was performed using one-way ANOVA followed by Tukey's Multiple Comparison Test.

Assessment of serum, liver, and kidney albumin by a multi-herbal formulation (SS-IM-21)

The mean serum, liver, and kidney albumin levels in the control and experimental groups of mice are shown in

Figure 2. The data show that as compared to the control, the mean blood levels of liver and kidney albumin in CCl_4-intoxicated mice were considerably lower ($P < 0.001$). When compared to mice treated with CCl_4, pre-treatment with the multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day dramatically accelerated the decline in albumin levels. When compared to the control group, 28 days of treatment with the newly created multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day did not demonstrate any appreciable variations in serum, liver, or kidney albumin levels.

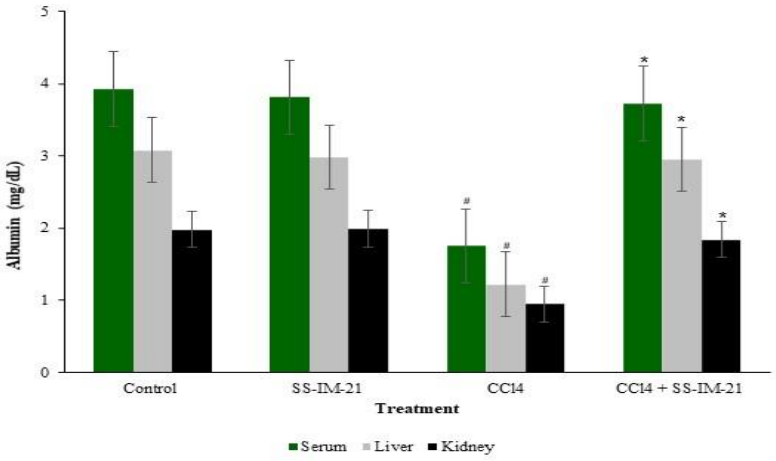

Figure 2. Effect of newly developed multi-herbal formulation (SS-IM-21) on albumin levels in mice. All data were expressed as means± SE (n = 6/group). #Significantly different from the control group at $P < 0.001$ and *Significantly different from (CCl_4) group values at $P < 0.001$. Data comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test.

Assessment of serum, liver, and kidney globulin by a multi-herbal formulation (SS-IM-21)

The mean serum, liver, and kidney globulin levels in the control and experimental groups of mice are shown in **Figure 3**. According to the data, mice intoxicated with CCl_4 had considerably lower mean serum levels of liver and kidney globulin than the control group ($P < 0.001$). When compared to mice treated with CCl_4, pre-treatment with the multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day significantly accelerated the decline in globulin levels. When compared to the control group, 28 days of treatment with the newly created multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day did not demonstrate any appreciable variations in serum, liver, or kidney globulin levels.

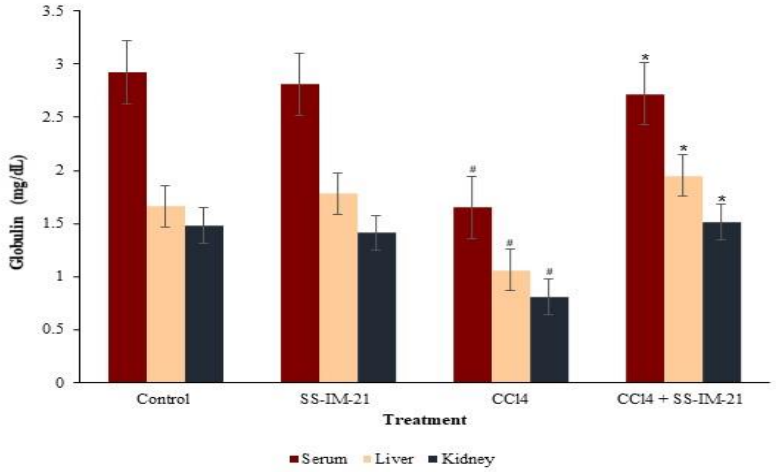

Figure 3. Effect of newly developed multi-herbal formulation (SS-IM-21) on globulin levels in mice. All data were expressed as means ± SE (n = 6/group). #Significantly different from the control group at $P < 0.001$ and *Significantly different from (CCl_4) group values at $P < 0.001$. Data comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test.

Assessment of serum, liver, and kidney albumin/globulin ratio by a multi-herbal formulation (SS-IM-21)

The mean serum, liver, and kidney albumin/globulin ratios for the control and experimental groups of mice are displayed in **Table 3**. Data show that when compared to the control, CCl_4-intoxicated mice had considerably lower mean serum liver and kidney albumin/globulin ratios ($P < 0.001$). When compared to mice treated with CCl_4, pre-treatment with the multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day significantly accelerated the drop in albumin/globulin ratio levels. When compared to the control group, 28 days of treatment with the newly created multi-herbal formulation (SS-IM-21) at a dose of 400 mg/kg/day did not demonstrate any appreciable variations in serum, liver, or kidney albumin/globulin ratio levels.

Table 3. Effect of SS-IM-21 on serum, liver, and kidney albumin/globulin in CCl_4-induced toxicity

Groups	Albumin/Globulin ratio		
	Serum	Liver	Kidney
Control	1.41 ± 0.14	1.49 ± 0.12	1.50 ± 0.15
SS-IM-21	1.44 ± 0.15	1.38 ± 0.14	1.43 ± 0.12
CCl_4	1.86 ± 0.12	1.59 ± 0.16	1.58 ± 0.16
CCl_4 + SS-IM-21	1.32 ± 0.13	1.41 ± 0.17	1.49 ± 0.16

The therapeutic benefits of medicinal plants are mostly caused by a variety of secondary metabolites [20]. Due to the synergistic effects of numerous plants, polyherbal medications are extremely helpful for treating a variety of disorders [21]. The primary pharmacological actions and the prevention of oxidative stress are carried out by the phenolic compounds and flavonoids found in aromatic plants [22]. Our research shows that administering CCl_4 inhibits normal body growth, food intake, and water intake. Co-administration of our formulation helped to maintain body weight, food, and water intake.

Normal body growth and development are made possible by protein. When an individual is exposed to an environmental contaminant, abnormal protein levels can stop the body from growing [23, 24]. A measure of liver and kidney damage is total serum protein [25]. The serum, liver, and kidney protein levels were shown to be dramatically reduced by carbon tetra chloride (CCl_4) in the current investigation. The serum, liver, and kidney protein levels were kept normal by co-administration with SS-IM-21.

Albumin is essential for maintaining the body's physiological functions [26, 27]. Given that it is produced by liver cells, it is one of the liver biomarkers. Poor nutrition is caused by low albumin levels [15, 28-33]. In this investigation, we found that mice pre-treated with the novel multi-herbal formulation (SS-IM-21) before chronic CCl_4 treatment recovered to normal albumin levels in serum, liver, and kidney. The findings demonstrate that SS-IM-21 is capable of preserving normal albumin levels in the presence of environmental toxins like CCl_4. However, a scientific investigation found that serum globulin has a role in chronic inflammation. Carbon tetra chloride (CCl_4) alters serum, liver, and kidney globulin and disturbs normal homeostasis, according to a recent study. Our research supports the fact that CCl_4 treatment reduced amounts of healthy globulin in the serum, liver, and kidney. Experimental animals' globulin levels were brought back to normal after treatment with the newly discovered formulation (SS-IM-21). The ratio of albumin to globulin further supports the protein change.

CONCLUSION

Carbon tetrachloride (CCl_4) was given to mice over an extended period, which inhibited their normal body growth and decreased their ability to consume food and water. This environmental toxin decreased serum and tissue levels of total protein, albumin, and globulin. Our newly created unique multi-herbal formulation may be able to keep critical protein levels at their normal levels and stop the harmful effects of CCl_4 on mice. Therefore, we think that the produced formulation made of medicinal herbs and medicinal spices may someday be used as a therapeutic drug.

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Conflict of interest: None

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Ethics statement: The Institutional Animal Ethics Committee (IAEC) of Dey's Medical Stores Manufacturing Limited gave its approval to the experimental methods (Approval No.

261/Dey's/IAEC/Pharma/2018) in their 61st meeting approved by CCSEA, Government of India.

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